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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 6 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Atrazine (080803), Reregistration Case No. 0062. Special Review. Ciba-Geigy Comments on the Triazine PD1; Additional Data on Metabolism in Corn and Sorghum.
CBRS No. 15633, DPBarcode No. D215509, MRID 43598629.

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Special Review of triazine herbicides, including atrazine, has been initiated (59 FR 60412, 11/23/94, PD1). Ciba-Geigy Corporation has submitted comments in response, including additional data on the nature of the residue in corn and sorghum; the present submission represents Volume 30 of registrant's comments. Assignment instructions are to review the present submission in response to the PD1 and provide evaluation for PD2/3. Conclusions below pertain only to the present submission, and its relation to the Agency position in the PD1.

Tolerances are established for residues of the herbicide atrazine, 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, in or on agricultural commodities (40 CFR 180.220(a)), and for combined residues of atrazine and its metabolites 2-amino-4-chloro-6-ethylamino-*s*-triazine, 2-amino-4-chloro-6-isopropylamino-*s*-triazine, and

2-chloro-4,6-diamino-*s*-triazine, in or on specified plant commodities (40 CFR 180.220(b)). Designations for the metabolites in the tolerance expression are G-28279, G-30033, and G-28273, respectively; structures are indicated in Figure 1. Atrazine is a List A Chemical. The Residue Chemistry Chapter to the Registration Standard was issued 7/25/83; the Registration Standard (Guidance Document) was issued 9/85; a Second Round Review (SRR) Residue Chemistry Chapter was issued 10/18/88.

Conclusions on This Submission

1. The previous submission on corn and sorghum demonstrated that metabolism of atrazine was extensive. Residues identified in corn and sorghum were parent, chloro, and hydroxy metabolites (see center and right side of Figure 1), and also a lanthionine conjugate in sorghum. (CBRS 10980, 6/3/93, J. Abbotts)

2. In the present submission on sorghum, the atrazine-lanthionine conjugate was isolated, and registrant identified additional atrazine conjugates with lanthionine sulfoxide and glutamine. Each of these conjugates individually represented 4% TRR or less.

3. With Extraction Method III, an acid autoclave procedure, from 52% to 76% of the TRR in corn and sorghum commodities was converted to cyanuric acid and the atrazine hydroxy metabolites G-34048, G-17794, and G-17792. This observation indicates that the position of the HED Metabolism Committee, that TRR should represent total residues containing the triazine ring, is a reasonable assumption.

4. Extraction Method III converted a major portion of TRR in corn and sorghum commodities to four hydroxy triazine compounds, but there appear to be limitations in using this technique to develop an enforcement method for determining total triazine ring residues, and registrant has not proposed such method development.

Conclusion with Regard to the PD1

The Agency position can be summarized in the following manner: Atrazine metabolism in plants is extensive, no single metabolite represents a large portion of the total triazine residue, analytical methods to measure total triazine ring residues are not available, and therefore, total radioactive residues from radiolabel field studies are the most appropriate data to use for risk assessment. The present submission on metabolism in corn and sorghum does not contradict the Agency position, and in fact reinforces it.

2

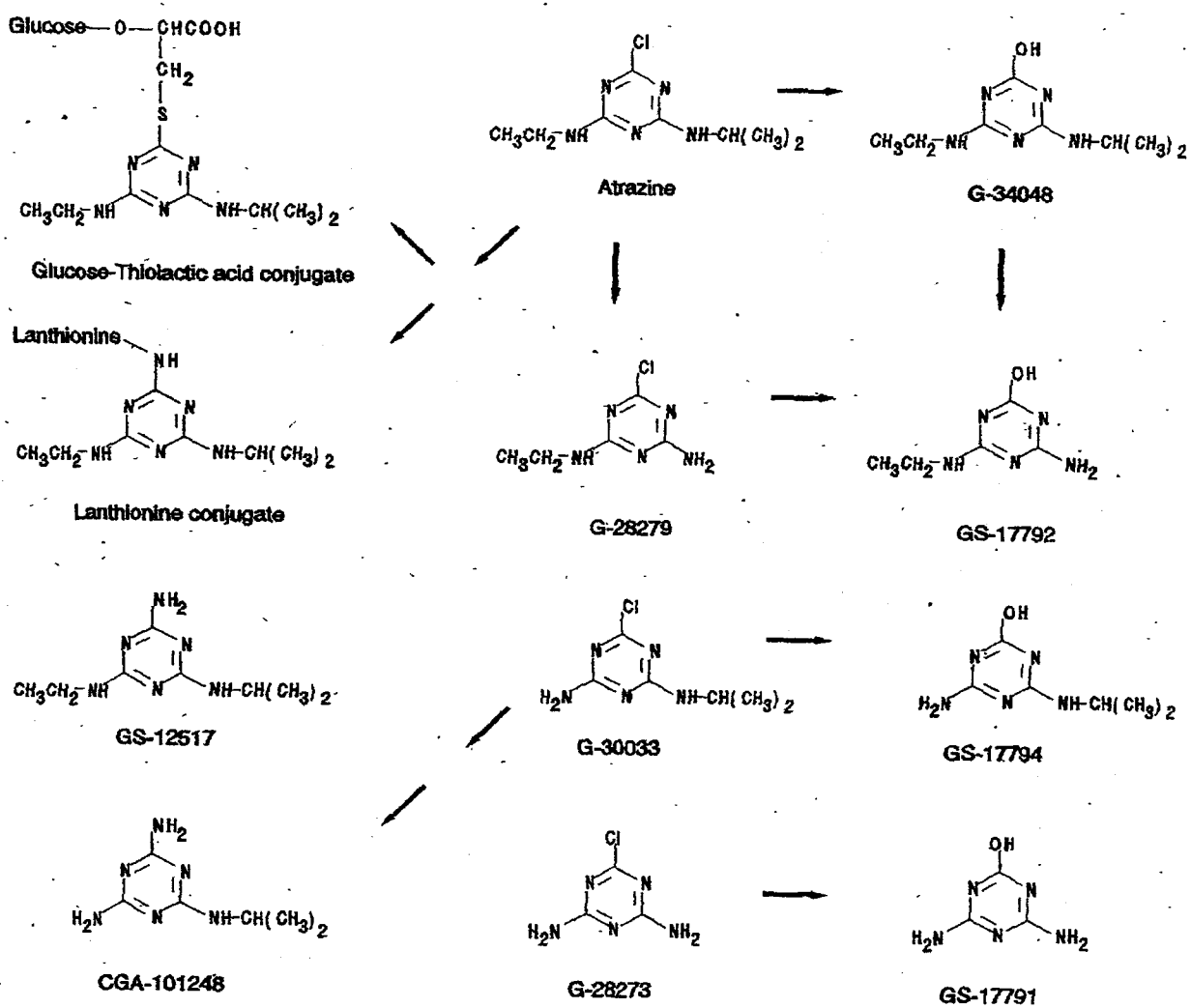


Figure 1. Atrazine metabolites identified in plants.

DETAILED CONSIDERATIONS

PD1 Position on Plant Metabolism

The initiation of special review on the triazines describes the Agency's position pertaining to triazine metabolism and residues of concern. The full FR notice contains more detail, but the following excerpts outline the Agency position (59 FR 60412, 11/23/94):

"In estimating triazine dietary risks, the Agency assumes that the total toxic residue of concern is the parent triazine compound plus all metabolites with a triazine ring, including among others, all chloro and hydroxy metabolites."

"In plants, atrazine and simazine are metabolized to numerous metabolites, no one of which has yet been shown to comprise a large portion of the total terminal residue.... Most metabolites have been shown to contain the intact triazine ring." (59 FR 60418)

"Based on its assessment of the structure-activity relationship and potential carcinogenicity of all registered triazine compounds, EPA believes metabolites which have been dechlorinated may be less potent carcinogens than the parent compounds.... However, in the absence of completed laboratory studies of the hydroxy metabolites, the Agency has relied on its equivalency policy and has made the assumption that all metabolites containing the triazine ring are equipotent as carcinogens as the parent compound when conducting its risk assessment." (59 FR 60418-60419)

"Since the registrants have been unable to develop analytical methodology which measures total triazine ring residues in non-radiolabel field trials, radiolabel field studies currently provide the best data to use for risk assessment. New radiolabel field studies for major dietary risk contributors for both atrazine and simazine have been submitted to the Agency and are currently under review." (59 FR 60419)

The Agency position can be summarized in the following manner: atrazine metabolism in plants is extensive, no single metabolite represents a large portion of the total triazine residue, analytical methods to measure total triazine ring residues are not available, and therefore, total radioactive residues from radiolabel field studies are the most appropriate data to use for risk assessment. The present submission will be reviewed with regard to this Agency position.

Background

In response to an Agency DCI of 10/90 requiring radiolabel field studies, the Registrant submitted field metabolism studies in corn and sorghum. Review concluded that no data for corn or sorghum were provided which alter the position of the HED Metabolism Committee that exposure assessment for atrazine should be conducted on the basis of total radioactive residues (CBRS 10980, 6/3/93, J. Abbotts). Residues identified in corn and sorghum were parent, chloro, and hydroxy metabolites (see center and right side of Figure 1), and also the lanthionine conjugate in sorghum. Subsequent to initiation of special review, and with the submission of protocols and additional data on storage conditions, the data on corn and sorghum metabolism were found acceptable. However, the conclusions of the previous review, including the position that exposure assessment should be based on total radioactive residues, remained in effect (CBRS 13059, 5/22/95, J. Abbotts).

As part of its response (Volume 30 of 54) to the FR notice initiating special review, the Registrant submitted the following document:

^{14}C-Atrazine: Nature of the Residue in Corn and Sorghum, Amendment No. 2 to the Final Report, Study Completed on 10/26/92, Ciba-Geigy Corporation, Greensboro, NC (MRID 43598629).

The performing laboratory for the original study, as well as for this amendment, was Hazelton Wisconsin, Inc., Madison, WI. Amendment No. 2, the present submission, describes additional laboratory work pertaining to extraction methods and the identification of residues.

Additional Laboratory Analysis

In the original metabolism study, corn and sorghum samples were extracted by two similar methods. In Method II, outlined in Figure 2, initial extraction was by refluxing with 0.5N HCl in methanol for approximately 1.5 h. Extraction Method I was similar, except that the initial extraction was with methanol:water (80:20).

Subsequent to the original corn and sorghum metabolism study, the Registrant found that with an acid autoclave extraction method, approximately two-thirds of the total radioactive residue in sugarcane was converted to known hydroxy metabolites containing the triazine ring (CBRS 12889, 6/29/95, J. Abbotts). In the present submission, extraction methods more vigorous than Methods I and II in the original study were tested for their ability to extract radioactive residues and convert them to known compounds.

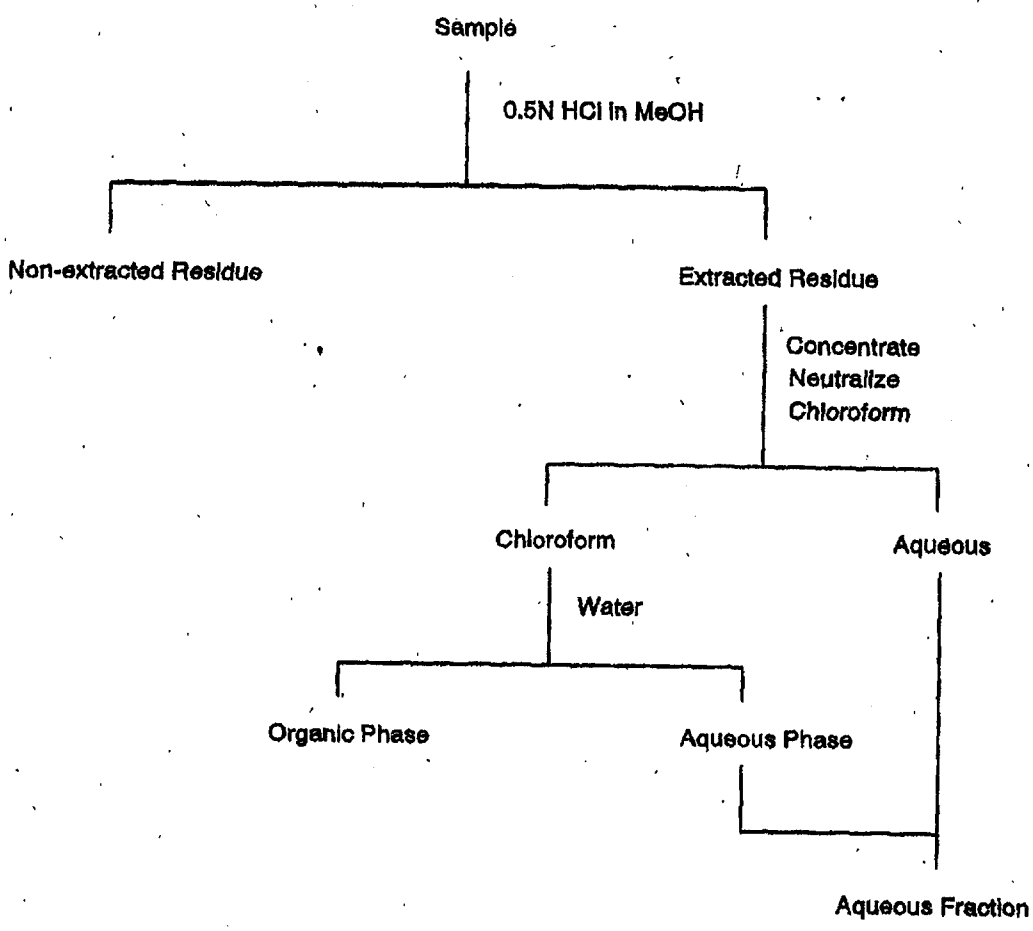


Figure 2. Extraction Method II for corn and sorghum (Reproduced from CBRS 10980, 6/3/93, J. Abbotts)

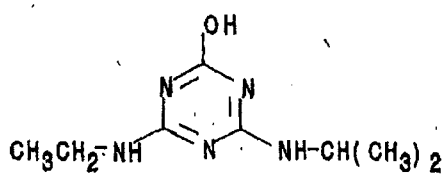
NY sorghum fodder was extracted by several different procedures for the initial step: Samples were extracted by reflux in 0.5N or 1.0N HCl, for 8, 16, or 72 hours, for a total of six different combinations. In another procedure, samples were initially extracted by 0.5N HCl in methanol (similar to the original Method II), followed by reflux of the aqueous phase in 1.0N HCl for 72 h. In another procedure, samples were extracted by sonication for 4.5 h in 0.5N HCl. In another procedure, samples were extracted in 0.5N HCl in an autoclave at approximately 120°C for 4.5 h or 24 h; the latter procedure, similar to that used previously with sugarcane, was designated Method III. Results of the different extraction protocols are summarized in Table 1:

Table 1. Extraction of TRR from NY mature sorghum fodder.

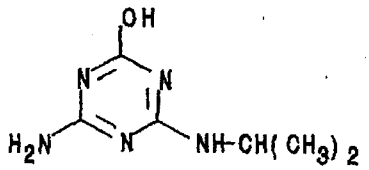
Extraction Method	HCl	% TRR Extracted as:		
		Organic	Aqueous	Total Extracted
Method I, aqueous methanol	None	10.0	48.5	58.5
8 h reflux	0.5 N	1.9	71.3	73.2
16 h reflux	0.5 N	2.5	75.6	78.1
72 h reflux	0.5 N	2.2	77.8	80.0
8 h reflux	1.0 N	2.3	71.2	73.5
16 h reflux	1.0 N	2.5	70.5	73.0
72 h reflux	1.0 N	1.0	76.6	77.6
4.5 h sonication	0.5 N	2.8	81.0	83.8
4.5 h autoclave	0.5 N	1.3	87.5	88.8
24 h autoclave (Method III)	0.5 N	1.1	82.0	83.1
1.5 h reflux, then 72 h reflux of aqueous phase	0.5 N with MeOH, then 1.0 N	11.9	67.4	79.3

Table notes: TRR was 1.043 ppm. Data on Method I were taken from MRID 42547116, reviewed in CBRS 10980, 6/3/93.

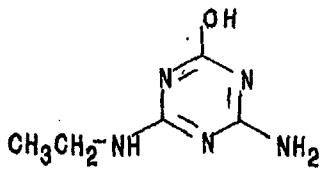
Aqueous fractions from each procedure were analyzed by Aminex A-4 cation exchange chromatography. The hydroxy triazine compounds shown in Figure 3 were detected with all procedures; detections of the amino triazines GS-12517 and CGA-101248 (see Figure 1) were also reported. Table 2 summarizes the assignment of residues:



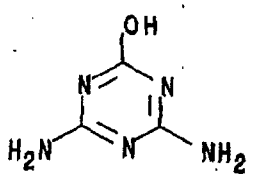
G-34048



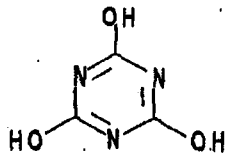
GS-17794



GS-17792



GS-17791



Cyanuric Acid

Figure 3. Residues detected after Extraction Method III

8

Table 2. Assignment of residues in NY mature sorghum fodder with extraction method.

Method	% TRR Assigned as:							
	Cyanuric acid	GS-17791	GS-17792	GS-17794	CGA-101248	G-34048	GS-12517	Total Assigned
8 h reflux, 0.5 N HCl	5.9	4.6	1.7	10.4	1.0	18.5	5.1	47.2
16 h reflux, 0.5 N HCl	6.3	5.9	1.9	11.5	1.6	20.2	8.3	55.7
72 h reflux, 0.5 N HCl	5.7	4.7	1.6	13.5	1.2	28.5	4.4	59.6
8 h reflux, 1.0 N HCl	5.0	5.5	1.6	9.4	1.9	15.1	6.2	44.7
16 h reflux, 1.0 N HCl	3.9	6.1	1.5	9.2	1.5	14.5	7.2	43.9
72 h reflux, 1.0 N HCl	8.9	4.8	2.2	11.1	0.4	30.3	1.8	59.5
4.5 h sonication in HCl	20.1	2.7	2.0	7.8	4.0	4.6	2.6	43.8
4.5 h autoclave in HCl	9.3	3.8	1.7	13.5	1.0	32.2	3.7	65.2
24 h autoclave in HCl (Method III)	17.5	0.8	1.2	8.5		37.5	0.3	65.8
1.5 h reflux in acid MeOH, then 72 h acid reflux of aqueous phase	8.2	1.3	1.2	10.3		25.0	1.0	47.0

Table notes: See Figures 1 and 3 for structures; blank spaces indicate residues not detected.

CBRS 15633, PD1 Comment, Atrazine, Corn and Sorghum, p. 10 of 14

The Registrant noted that in its previous submission on corn and sorghum metabolism, with Extraction Method I, 13.7% TRR in NY sorghum fodder was identified (CBRS 10980, 6/3/93, J. Abbotts). All of the extraction methods in Table 2 resulted in a significant increase in the % TRR identified. With Method III, the highest portion of the total residue, 65.8% TRR, was assigned to residues containing the triazine ring. Accordingly, the Registrant then used Method III to extract other NY corn and sorghum samples, and the aqueous extract was analyzed for the hydroxy triazines shown in Figure 3. Assignment of residues in each sample is summarized in Table 3:

Table 3. Assignment of residues in NY corn and sorghum samples with Extraction Method III.

Residue	% TRR Assigned in Corn [100% TRR in ppm]:			
	30 DAT Forage [2.840]	Silage Stage Forage [0.499]	Mature Fodder [1.549]	Mature Grain [0.034]
Cyanuric acid	8.7	17.8	20.2	38.8
GS-17791			0.4	1.2
GS-17792	1.6	2.3	1.7	2.1
GS-17794	6.6	13.5	13.0	8.4
G-34048	59.5	32.7	28.3	2.2
Total	76.4	66.3	63.6	52.7
Residue	% TRR Assigned in Sorghum [100% TRR in ppm]:			
	30 DAT Forage [5.351]	Silage Stage Forage [1.071]	Mature Fodder [1.043]	Mature Grain [0.033]
Cyanuric acid	4.9	16.7	13.0	38.9
GS-17791	0.5	0.8	0.8	8.1
GS-17792	1.6	1.7	1.0	0.9
GS-17794	4.6	8.3	7.7	3.6
G-34048	58.1	41.3	29.8	2.8
Total	69.7	68.8	52.3	54.3

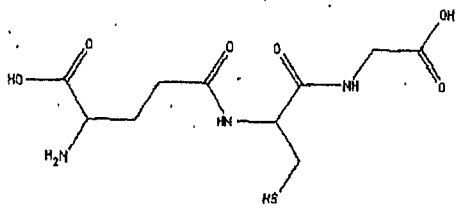
Table notes: DAT=days after treatment. See Figure 3 for structures. Blank spaces indicate residues not detected.

In its previous submission on metabolism in sorghum, the Registrant had identified a lanthionine-atrazine conjugate as a

component of Peak No. 7 when aqueous fractions were analyzed by Aminex A-4 ion exchange chromatography. The Registrant did not assign % TRR values to the lanthionine conjugate, however, because data indicated that Aminex Peak 7 might contain other metabolites. (CBRS 10980, 6/3/93, J. Abbotts) In the present submission, the Registrant has performed additional work on this peak.

Aqueous fractions obtained with Extraction Method I from seven extractions of NY sorghum forage collected 30 days after treatment (DAT) were combined and analyzed by Aminex A-4 chromatography. Peak 7 was collected, ammonium formate was removed by additional Aminex A-4 chromatography, and Peak 7 was then divided into three major peaks 7A, 7B/C, and 7D by HPLC Method III, using a YMC-Pack AQ-323 ODS column, eluted with gradients in 1% acetic acid in water (Solvent A) and 1% acetic acid in acetonitrile (Solvent B). Each of these three major peaks was further purified by HPLC and/or thin layer chromatography (TLC), and then analyzed by liquid chromatography/ mass spectrometry.

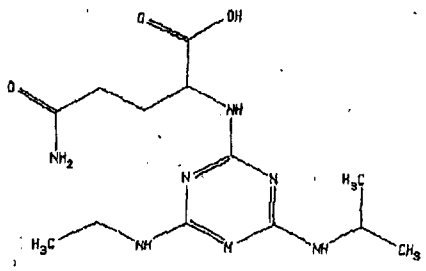
Peak 7A represented 4.0% TRR, and its mass spectrum was consistent with the lanthionine-atrazine conjugate previously identified. Peak 7B/C was split into two peaks by HPLC Method VII, using the same column and Solvents as HPLC Method III, but varying the gradient conditions. These two peaks combined represented 1.0% TRR, and their mass spectra were consistent with stereoisomers of a lanthionine-sulfoxide conjugate. Peak 7D represented 0.5% TRR, and its mass spectrum was consistent with an atrazine-glutamine conjugate. Structures of each of the three conjugates are given in Figure 4. The Registrant proposed that these residues were formed by conjugation of atrazine with glutathione, and then rearrangement and modification of the glutathione portion of the conjugate. Proposed intermediates in this glutathione pathway were not identified, and are designated by multiple arrows in Figures 1 and 4.

Atrazine + 

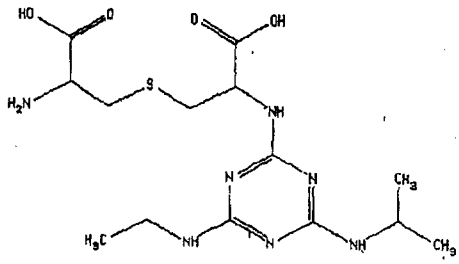
Glutathione

[Conjugation, rearrangement, and/or additional metabolism]

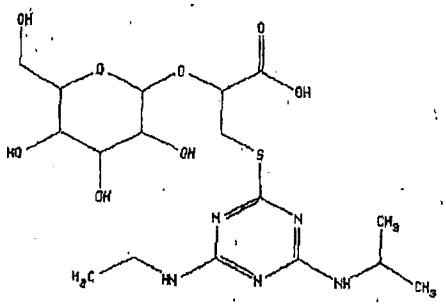
GS-12517



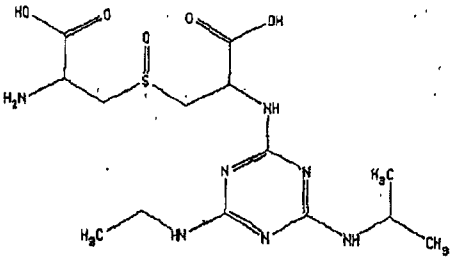
Atrazine-glutamine conjugate



Atrazine-lanthionine conjugate



Atrazine-glucose-thiolactic acid conjugate



Atrazine-lanthionine sulfoxide conjugate

Figure 4. Residues identified in proposed pathway for glutathione conjugation.
(See Figure 1 for structures not shown here)

CBRS Comments, Additional Laboratory Work

Conclusion 1: The previous submission on corn and sorghum demonstrated that metabolism of atrazine was extensive. Residues identified in corn and sorghum were parent, chloro, and hydroxy metabolites (see center and right side of Figure 1), and also a lanthionine conjugate in sorghum. (CBRS 10980, 6/3/93, J. Abbotts)

Conclusion 2: In the present submission for sorghum, the atrazine-lanthionine conjugate was isolated, and the Registrant identified additional atrazine conjugates with lanthionine sulfoxide and glutamine. Each of these conjugates individually represented 4% TRR or less.

Conclusion 3: With Extraction Method III, an acid autoclave procedure, from 52% to 76% of the TRR in corn and sorghum commodities was converted to cyanuric acid and the atrazine hydroxy metabolites G-34048, G-17794, and G-17792. This observation indicates that the position of the HED Metabolism Committee, that TRR should represent total residues containing the triazine ring, is a reasonable assumption.

Despite the ability to convert residues to a few metabolites, Extraction Method III presents limitations in applying it to an enforcement method for total triazine ring residues. Previous review has noted that although cyanuric acid represents a common moiety, its detection does not distinguish between atrazine and other triazine herbicides as the original source of residues; in addition, cyanuric acid represents background levels on the order of 1 ppm in plant and animal commodities (CBRS 9167, 1/22/92, M.S. Metzger).

There also appear to be limitations in using Extraction Method III for method development by detecting hydroxy triazine compounds other than cyanuric acid. In a previous submission, the Registrant proposed using detection of G-34048 and GS-17794 as a marker method in corn and sorghum. The limit of determination for each metabolite was the lowest level analyzed, 0.02 ppm in corn and sorghum commodities (CBRS 10980, 6/3/93, J. Abbotts). The combined limit of determination is greater than the TRR in mature grain of corn or sorghum from the NY samples, and even with Extraction Method III, the metabolites G-34048 and GS-17794 each represent less than 10% TRR in these grain samples (see Table 3). We note that the Registrant has not proposed development of an enforcement method using Extraction Method III.

Conclusion 4: Extraction Method III converted a major portion of TRR in corn and sorghum commodities to four hydroxy triazine compounds, but there appear to be limitations in using this technique to develop an enforcement method for determining total

13

triazine ring residues, and registrant has not proposed such method development.

The Agency's position in the PD1 was described and summarized above (see section on PD1 Position on Plant Metabolism). The present submission does not contradict the Agency's position, and in fact reinforces it, leading to the following overall conclusion:

Conclusion with Regard to the PD1: The Agency position can be summarized in the following manner: Atrazine metabolism in plants is extensive, no single metabolite represents a large portion of the total triazine residue, analytical methods to measure total triazine ring residues are not available, and therefore, total radioactive residues from radiolabel field studies are the most appropriate data to use for risk assessment. The present submission on metabolism in corn and sorghum does not contradict the Agency position, and in fact reinforces it.

cc:Circ, Abbotts, RF, Atrazine List A File, SF
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JA12\atrazine.9